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Nrf2 activation protects cells from HEMA-induced apoptosis

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Dental resin monomers like HEMA (2-hydroxylethyl methacrylate) exert cytotoxicity *via* apoptosis which is causally related to the elevated production of ROS (reactive oxygen species) [1]. Here, we hypothesized that the activation of the redox-sensitive transcription factor Nrf2 [2] as an antioxidant cell response counteracts HEMA-induced cell death through the Nrf2-regulated expression of enzymatic antioxidants. RAW264.7 mouse macrophages were preincubated with t-BHQ (*t*-butyl-hydroquinone) (0-50μM) which is a classical inducer of the Nrf2 pathway. Then, cells were exposed to HEMA (0-8 mM) for 24h with or without t-BHQ. Expression of Nrf2 and Nrf2-regulated enzymatic antioxidants (catalase, peroxiredoxin, thioredoxin 1, thioredoxin reductase, heme oxygenase-1, glucose 6-phosphate dehydrogenase, transaldolase) was detected by Western blotting. The percentage of cells undergoing apoptosis or necrosis was determined by flow cytometry after Annexin-V-FITC/propidium iodide (PI) staining. Differences between medians (plus 25%/75% percentiles) were statistically analysed (Mann-Whitney-U test). The expression of Nrf2 and Nrf2-regulated enzymatic antioxidants increased in cell cultures exposed to t-BHQ and was further enhanced by HEMA. The number of viable cells detected in the untreated samples (97%) significantly decreased in a concentration-dependent manner in cultures exposed to HEMA. About 61% viable cells were found after exposure to 8 mM HEMA along with the increased number of cells in the various phases of cell death. However, the presence of t-BHQ protected cells from HEMA-induced cell death through a shift from necrotic cells (PI-stained) and late apoptotic (Annexin-V+PI) to the early apoptotic ones (Annexin-V). The number of cells in late apoptosis and necrosis significantly decreased about 4-fold in cultures treated with 8mM HEMA in the presence of 50μM t-BHQ compared to cultures exposed to 8 mM HEMA alone. These results provide evidence that the increased expression of Nrf2 and Nrf2-regulated enzymatic antioxidants is an adaptive cell response to inhibit HEMA-induced cell death.

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References

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Keywords
Nrf2; oxidative stress; apoptosis; tBHQ; HEMA.